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RESPONSES OF SKELETAL MUSCLE TO UNLOADING - A REVIEW

Marc E. Tischler, Stephen R. Jaspers, Erik J. Henriksen and Stephan Jacob

Department of Biochemistry
University of Arizona
Tucson, AZ 85724

Suspension models have been used to study muscle response to reduced activity. During 6 days of tail-casting, the soleus (SOL) atrophies while the extensor digitorum longus grows relatively normally. After discounting those changes in both muscles due primarily to increased secretion of adrenal hormones, the following conclusions regarding the specific responses of the SOL could be drawn. 1) Atrophy is probably due primarily to increased protein degradation. 2) Decreased synthesis of glutamine may result from reduced availability of ammonia due to diminished use of ATP. 3) Greater muscle glycogen seems to reflect an increased response to insulin of glucose uptake which leads to greater glucose metabolism. 4) Faster catabolism of branched-chain amino acids can be attributed to enhanced flux through ketoacid dehydrogenase. Studies by others using tail-casted suspended rats showed in the SOL: 1) a gradual switch from type I to type II fibers, 2) increased acid protease activity and 3) altered muscle function and contractile duration. Using harness suspended rats, others showed in the SOL: 1) significant atrophy, 2) increased numbers of glucocorticoid receptors and 3) no change in muscle fatigability.

Our studies have focused primarily on the metabolic responses of skeletal muscle to unloading. Some of these results have been reported previously (1-5) or in this journal (6). Unloading of the rat hindlimbs in these studies is accomplished by tail-cast suspension as described elsewhere (1) by a method adapted from Morey (7). Another model used for studying the response of muscle to unloading is that of Musacchia and coworkers who utilize a harness suspension system (8). A major advantage of this system is that it permits the investigator to also test the effect of antiorthostasis (head-down tilt), which is not feasible with the current tail-cast model. One important advantage of the tail-cast model is that the animals continue to grow (1) while harness-suspended animals may lose weight (8). This finding suggests that the harness-suspended animals may be under more stress than the tail-casted animals. Despite these differences, both models yield a similar pattern of muscle atrophy and altered muscle growth. The soleus is most responsive followed by the gastrocnemius and plantaris, with the extensor digitorum longus and tibialis anterior being generally resistant to the effects of unloading. This is not surprising since the posterior muscles are load-bearing while the anterior muscles are non-load-bearing. Recent studies of muscles obtained from rats flown on the SL-3 mission showed some similar biochemical responses and differences in size as those muscles from suspended rats (9,10).

STUDIES FROM THIS LABORATORY

Atrophy and Growth Failure of Rat Hindlimb Muscles. Atrophy and growth failure of muscle in the tail-cast suspension model was evaluated in hindlimbs of female Sprague-Dawley rats. Based on measurements of food consumption, animal growth rate, urinary excretion of urea and ammonia, and muscle size, 6 days seemed to be the optimum duration of suspension for studying muscle unloading. After 6 days, the soleus, plantaris and gastrocnemius muscles from suspended animals were 27, 10, and 11% smaller, respectively, than those from tail-casted weight-bearing animals. The extensor digitorum longus and tibialis anterior muscles generally were affected only slightly by suspension (≤6 days). Wet weight-to-dry weight ratios were smaller in the plantaris (-0.19) and gastrocnemius (-0.19) muscles from suspended rats. In the plantaris this difference coincided with a higher protein concentration (+12 mg/g). In vitro protein metabolism in the soleus of suspended rats showed both slower protein synthesis and faster protein degradation, whereas these processes were unaltered in the extensor digitorum longus.

Role of Glucocorticoids in Atrophy of the Soleus. This problem was studied during 6 days of suspension using adrenalectomized rats. Adrenalectomy did not prevent this atrophy. After tail-casting, administration of cortisol to control and suspended adrenalectomized animals produced a similar percent loss (13%-18%) of body weight and mass of the weight bearing soleus and extensor digitorum longus muscles. The unloaded soleus of cortisol-treated animals lost approximately 40% of its mass. Unloading of the soleus but not the extensor digitorum longus, was associated with an increased concentration only of sarcoplasmic proteins, while cortisol administration generally increased only the concentration of myofibrillar (+stromal) proteins in both muscles. Hence, these protein fractions respond differently to reduced activity and glucocorticoid treatment. Unloading of hindlimbs of adrenalectomized animals led to faster protein degradation, slower synthesis of sarcoplasmic proteins and faster synthesis of myofibrillar proteins in the isolated soleus and no differences for the extensor digitorum longus. For the cortisol-treated animals the difference, due to suspension, in synthesis of myofibrillar proteins was enhanced and of sarcoplasmic proteins was abolished in the soleus, while both soleus and extensor digitorum longus showed faster protein degradation. These results suggest that it is unlikely that any elevation in circulating glucocorticoids associated with tail-cast suspension are solely responsible for atrophy of the soleus, and that catabolic amounts of glucocorticoids may alter the response of muscles to unloading.

Amino Acid Metabolism in the Soleus. Amino acid metabolism was investigated in atrophied soleus from rats subjected to 6 days of tail-cast, hindlimb suspension. The fresh-frozen unloaded muscle showed higher concentrations of tyrosine and glutamate but lower amounts of aspartate, glutamine and ammonia and a lower ratio of

glutamine/glutamate than normal muscle. The atrophied muscle also showed faster in vitro production of alanine and tyrosine and slower utilization of glutamate and aspartate. Despite a greater activity of glutamine synthetase, synthesis of glutamine was slower in the soleus of suspended rats than in control muscle. Provision of ammonium chloride and/or glutamate showed that this slower synthesis of glutamine in the atrophied soleus probably was due to limiting amounts of free ammonia and not of glutamate. Flux through AMP deaminase was probably slower as demonstrated by the maintenance of a greater pool of total adenine nucleotides and by the slower release of nucleosides by the incubated soleus of suspended than of control rats. The extensor digitorum longus of suspended animals showed greater glutamine production, glutamine synthetase activity and aspartate utilization than control muscles. Data from muscles of intact, adrenalectomized non-treated and adrenalectomized cortisol-treated rats suggested that the greater glutamine synthetase activity was mediated possibly by higher circulating glucocorticoid hormones and a greater response of the soleus to these hormones. Glutamine synthesis in skeletal muscle may be regulated primarily by the availability of ammonia which is associated with the degradation of adenine nucleotides, and secondarily by the amount of glutamine synthetase and glutamate in the tissue.

Since branched-chain amino acids are degraded rapidly by muscle and this process increases with fasting and trauma, we measured their metabolism in the atrophied soleus. Irreversible decarboxylation of leucine, isoleucine and valine were enhanced markedly in the unloaded soleus and to a lesser extent in the extensor digitorum longus. Transamination of leucine was increased presumably by the mass action effect of accelerated decarboxylation of ketoisocaproic acid. The effect in the extensor digitorum longus but not in the soleus could be abolished by adrenalectomy. Therefore, faster metabolism of the branched-chain amino acids in the soleus is likely due to unloading per se rather than to adrenal hormones. Preliminary studies suggest that increased decarboxylation was due to greater total enzyme activity rather than a rise in the percent actual of total activity.

Glucose Metabolism in the Soleus. Glycogen content in the soleus from suspended rats was 77% greater than in these muscles from weight bearing animals. Carbohydrate metabolism in the presence and absence of 10^{-4}U/ml insulin was measured in vitro using a protocol in which muscles were glycogen-depleted by preincubation with isoproterenol. Glucose oxidation, release of lactate and pyruvate, and net turnover of glycogen were determined for the subsequent 1 hr incubation period. In the control soleus insulin increased glucose oxidation (119%) and lactate and pyruvate release (29%), and caused a net glycogen synthesis (1.50 nmol glucose/mg muscle). In the soleus of suspended animals, these values were 150%, 150%, and 7.09, respectively. Overall glucose utilization, as determined by these parameters, increased 77% in the control and 340% in the inactive SOL upon insulin stimulation. The extensor digitorum longus muscles from both groups of rats showed no differences. A dose response curve for insulin stimulation of deoxyglucose uptake also showed no differences for the extensor digitorum longus muscles. However, for the soleus the response to insulin at all concentrations tested (10^{-5} to 10^{-1} U/ml) was greatest in the unloaded muscle. These results suggest that the atrophied soleus is more sensitive to insulin than the control muscle, and that this sensitivity may account, in part, for the greater glycogen content in the less active muscle. Accordingly, the tissue levels of glucose-6-phosphate were greatest in the unloaded soleus. Since this metabolite can activate glycogen synthase$_d$, it could possibly contribute to the accumulation of glycogen with unloading.

Biochemical Response to Chronic Shortening of the Unloaded Soleus. One leg of tail-casted suspended rats was immobilized in a plantar-flexed position to test whether chronic shortening of posterior leg muscles affected the metabolic response to unloading. The immobilized plantaris and gastrocnemius muscles of these animals showed about 20% loss of muscle mass in contrast to simply a slower growth rate with unloading. Loss of mass of the soleus during suspension was not accentuated by chronic shortening. Although protein degradation in the SOL of the plantar-flexed limb was slightly faster than in the contralateral free limb, this difference was offset by faster synthesis of the myofibrillar protein fraction of the chronically shortened muscle. Total adenine nucleotides were 17% lower in the chronically shortened soleus following incubation. Glutamate, glutamine and alanine metabolism showed little response to chronic shortening. These results suggest that in the soleus chronic shortening did not alter significantly the metabolic responses to unloading and reduced activity.

Metabolism of Muscles from Dorsiflexed Hindlimbs. Protein, amino acid and purine nucleotide metabolism were studied following passive stretch of posterior muscles (soleus, plantaris and gastrocnemius) and chronic shortening of two anterior hindlimb muscles (extensor digitorum longus and tibialis anterior). Immobilization in dorsiflexion of the hindlimb of rats subjected to tail-cast suspension resulted in hypertrophy of the soleus, faster growth of the plantaris and gastrocnemius, and slower growth of the extensor digitorum longus and tibialis anterior muscles compared to those of the contralateral non-weight bearing free limb. Hypertrophy of the stretched soleus was associated with a greater concentration of RNA, faster protein synthesis, especially in the sarcoplasmic protein fraction, and slower protein degradation than the contralateral muscle. The stretched soleus also showed faster production of glutamine, slower production of alanine, greater utilization of aspartate and lower concentrations of adenine nucleotides than the soleus of the contralateral free limb. In contrast, chronic shortening and the resulting slower growth of the extensor digitorum longus muscle was associated with: a lower concentration of RNA and slower protein synthesis. No significant difference was observed in protein degradation or the production of glutamine, glutamate, alanine or aspartate, or in the concentration of adenine nucleotides in this muscle. Passive stretch of posterior muscles prevented or reversed those changes in muscle size and metabolism associated with hindlimb unloading while chronic shortening of the anterior muscles produced changes in muscle metabolism associated with muscle disuse.

STUDIES FROM OTHER LABORATORIES

Muscle Size and Protein Metabolism. In other studies using tail cast or harness suspension, unloading of the hindlimbs of rats or mice showed consistently the largest response by the soleus muscle (11-13). Using tail cast suspension, Morey-Holton and Wronski (11) found that after unloading, the soleus was 40 to 45% smaller, the gastrocnemius was 15 to 20% smaller and the extensor digitorum longus was 5 to 10% smaller than weight bearing controls, when normalized to body mass. Feller et al. (14) also found the maximal response of the soleus to be 45% and was reached by day 7 of suspension, the same length of time in which we achieve near maximal response in terms of percent difference (1). Using the harness-suspension model, Musacchia and coworkers (12) found after 1 week of suspension, a 29% difference from weight bearing controls which increased further to 42% by the end of week 2. While we reported smaller differences for the soleus (1), it is because we use tail-casted weight bearing animals as controls for the stress of casting. Comparison with a normal weight bearing group showed 34% and 42% differences at days 6 and 12 of suspension, respectively. The mouse soleus showed 29% difference after 1 or 2 weeks of harness suspension (13). In harness-suspended rats, like tail-casted animals, the gastrocnemius and plantaris are less responsive than the soleus (12,13). The same pattern is apparent in true hypogravity as in simulated weightlessness. Muscles of rats flown on Cosmos 986 showed 35%, 20% and 15% lower relative masses for the soleus, gastrocnemius and extensor digitorum longus than in control animals (15). We also found a similar pattern for rats flown on SL-3, as reported elsewhere in this journal (10).

One interesting observation is that the percent mass differences for the gastrocnemius and plantaris diminish beyond one week of harness-suspension. The differences for rat and mouse gastrocnemius drop from 19% after 1 week to 10% after 2 weeks and for the plantaris, there is no significant difference after 2 weeks suspension (12,13). In contrast with tail-casting, the percent difference remains the same or increases between 6 and 12 days of suspension (1).

The reduced mass of the soleus with unloading is due to diminished protein content since wet wt/dry wt ratios are not altered (1,13). In accord with our in vitro observation of faster protein degradation (1), Musacchia et al (12) reported increased urinary excretion of 3-methylhistidine, an in vivo indicator of muscle protein degradation. In contrast, Feller et al (14) found no increase in protein breakdown using muscle homogenates. However use of such measurements without intact cells is questionable. They did find that cell free synthesis of protein in unloaded muscle was up to 30-50% slower in accord with our observations for the soleus of small (<120 g) suspended rats (1). One other indication that protein breakdown may be increased is the presence of 32% more acid protease activity in the soleus by day 4 of tail cast suspension (16).

Physiological Parameters. To attempt to correlate changes in muscle function with muscle atrophy, other investigators have measured a number of physiological parameters. Comparison of the soleus of suspended rats (11) with those flown on Cosmos 986 (15) showed a marked diminution in maximum isometric tension (-50% and -40%, respectively). Templeton and coworkers (16) reported diminished contractile function and duration with increased contractile speed. The latter adaptation was attributed to a decrease in type I fibers from 70-90% to 50% after 4 weeks of suspension. After 3 weeks these investigators also found a fall in type I myosin enzyme. A similar study with the gastrocnemius showed no difference in contraction or one-half relaxation time (17). Contractile function was diminished as shown by dP/dt, and apparently type I (slow -twitch) fibers in this muscle showed a greater response to unloading than did fast-twitch fibers. In contrast to these findings, Fell et al. (18) reported that 1 week of harness-suspension did not affect the contractile properties of fast-twitch fibers in gastrocnemius or the slow-twitch soleus muscle. However, fatigability was increased generally in the gastrocnemius.

In unloaded soleus, gastrocnemius and plantaris of harness-suspended rats (19), as in immobilized skeletal muscles (20) the number of glucocorticoid receptors increases. Since these hormones have catabolic effects on muscle, it is possible that this increased binding capacity could provide an important mechanism for biochemical changes in the soleus. While increased responsiveness to glucocorticoids could explain in part the greater activity of glutamine synthetase in the soleus, it did not account for atrophy of the soleus or the increased metabolism of the branched-chain amino acids (5). It is possible, however, that increased binding of glucocorticoids could augment some of the responses of the soleus to unloading.

Recovery. Some studies have also focused on recovery from unloading. Elsewhere in this journal, we report the effects of 12 h recovery on some biochemical parameters in the soleus (9,10,21). Musacchia and coworkers showed that muscles of suspended rats recover mass rapidly following 7 or 14 days of harness-suspension (12). Templeton et al (16) reported that 7 days of recovery following a 2 week suspension was sufficient to return values for muscle wet weight, peak contraction force, one-half relaxation time and levels of myosin type I to near control values.

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